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Protective effect of ischemic preconditioning on ischemia/reperfusion-induced acute kidney injury through sympathetic nervous system in rats

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ABSTRACT

We have found that a series of brief renal ischemia and reperfusion (preconditioning), before the time of ischemia significantly attenuated the ischemia/reperfusion-induced acute kidney injury through endothelial nitric oxide synthase. In this study, we examined the effects of ischemic preconditioning on renal sympathetic nervous system and kidney function in ischemia/reperfusion-induced acute kidney injury with or without nitric oxide synthase inhibitor. Ischemia/reperfusion-induced acute kidney injury was made by clamping the left renal artery and vein for 45-min followed by reperfusion, 2 weeks after the contralateral nephrectomy. Ischemic preconditioning, consisting of three cycles of 2-min ischemia followed by 5-min reperfusion, was performed before the 45-min ischemia. Ischemic preconditioning suppressed the enhanced renal sympathetic nerve activity during ischemia and the elevated renal venous plasma norepinephrine level after reperfusion, and attenuated renal dysfunction and histological damage. The renoprotective effect of ischemic preconditioning was diminished by N^G-nitro-L-arginine methyl ester (0.3 mg/kg, i.v.), a nonselective nitric oxide synthase inhibitor, 5 min before the start of ischemic preconditioning. Thus, ischemic preconditioning decreased renal sympathetic nerve activity and norepinephrine release probably through activating nitric oxide production, thereby improving ischemia/reperfusion-induced acute kidney injury.

1. Introduction

Prior exposure to brief periods of tissue ischemia leads to a state of increased resistance to the effects of subsequent ischemia/reperfusion-induced injury. Phenomenon was referred to as ischemic preconditioning by Murry et al. (1986), who first demonstrated this protective effect in the dog heart. Furthermore, effects of ischemic preconditioning has been investigated in other organs, such as the brain (Heurteaux et al., 1995), the liver (Peralta et al., 1999), the skeletal muscle (Schroeder et al., 1996), and the kidney (Lee and Emala, 2000).

Brief ischemic preconditioning followed by ischemia and reperfusion, protects the kidney against ischemia/reperfusion-induced injury and hampers the progress of ischemia-related kidney diseases (Correa-Costa et al., 2012). In previous study, we reported that ischemic preconditioning treatment markedly suppresses norepinephrine and improves cardiac dysfunction after ischemia/reperfusion (Tawa et al., 2010), however, the role of renal sympathetic nerve activity in ischemic preconditioning has not been reported. In the kidney, we demonstrated that pretreatment with FK409, a spontaneous NO releaser, has a powerful renoprotective action against ischemia/reperfusion-induced renal dysfunction and histological damage (Matsumura et al., 1998), while pretreatment with N^G-nitro-L-arginine methyl ester (L-NAME), a non-selective NO synthase inhibitor, aggravated the ischemia/reperfusion-induced renal damage (Kurata et al., 2005). We noted that the protective effect of ischemic preconditioning on ischemia/reperfusion-induced acute kidney injury is associated with renal NO production following the increase in endothelial NO synthase protein expression after reperfusion (Yamashita et al., 2003). Moreover, the protective effect of ischemic preconditioning on ischemia/reperfusion-induced renal dysfunction and histological damage was not observed in endothelial NO synthase-deficient mice (Yamasowa et al., 2005).

In the present study, we evaluated whether the renoprotective effect of ischemic preconditioning on the ischemia/reperfusion-induced acute kidney injury is attributed to renal sympathoinhibitory effects as well as nitric oxide production.

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2. Materials and methods

2.1. Animals and experimental design

Male Sprague-Dawley rats (10 weeks of age, Japan SLC, Shizuoka, Japan) were used. The animals were housed in a light-controlled room with a 12-h light/dark cycle and were allowed ad libitum access to food and water. All animals were treated according to the procedures approved by the Experimental Animal Committee at Osaka University of Pharmaceutical Sciences (Osaka, Japan).

To develop renal ischemic model, the rats were anesthetized with pentobarbital (50 mg/kg body weight) and the right kidney was removed through a small flank incision, 2 weeks before the study (at 8 weeks of age). After a 2-weeks recovery period, uninephrectomized rats were divided into four groups: (1) sham-operated control, (2) untreated ischemic acute kidney injury, (3) ischemic acute kidney injury treated with ischemic preconditioning, (4) ischemic acute kidney injury treated with ischemic preconditioning and L-NAME. Six animals were provided for each group (total animals: $n=24$). The rats were anesthetized with pentobarbital (50 mg/kg, i.p.), and the left kidney was exposed through a small flank incision. The left renal artery and vein were occluded with a nontraumatic clamp for 45 min. At the end of the ischemic period, the clamp was released to allow reperfusion. Ischemic preconditioning comprised of three cycles of 2-min ischemia followed by 5-min reperfusion, was performed before the 45-min ischemia. Ischemic period, interval, and cycle were determined, according to the method previously reported from our laboratory (Yamashita et al., 2003). L-NAME (0.3 mg/kg) or vehicle (0.9% saline) was injected 5 min before the start of ischemic preconditioning, into the left external jugular vein in a volume of 1 ml/kg. The animals exposed to 45-min ischemia were housed in metabolic cages at 24 h after reperfusion and 5-h urine samples were collected. Blood samples were drawn from the abdominal aorta at the end of the urine collection period under pentobarbital anesthesia (50 mg/kg, i.p.). The plasma was separated by centrifugation at $1700\times g$ for 15 min at 4 °C. These samples were used for measurement of renal functional parameters. The left kidneys were excised and used for light microscopic observation.

In separate experiments, we examined the effect of ischemic preconditioning on changes of norepinephrine level in renal venous plasma after reperfusion. Under pentobarbital (50 mg/kg, i.p.) anesthesia, an abdominal midline incision of the uninephrectomized rats was made, and the left kidney was exposed. A curved 26-gauge needle was inserted into the left renal vein for venous blood sampling. Each blood sample was taken from sham-operated control and rats with or without ischemic preconditioning, immediately after the reperfusion or 1 day after reperfusion following 45-min ischemia. The sampling period (one sampling from each animal) was 2 min in duration. Plasma was immediately separated by centrifugation at $1700\times g$ for 15 min at 4 °C. These samples were stored at −80 °C until the assay for norepinephrine concentration. Six animals were provided for each group (total animals: $n=42$).

2.2. Renal nerve recording

In another set of experiments, electrical signals of renal neural activity were directly recorded for evaluation of changes in renal sympathetic nerve activity during the 45-min ischemic period. In this experiment, uninephrectomized rats were divided into three groups: (1) untreated ischemic acute kidney injury, (2) ischemic acute kidney injury treated with ischemic preconditioning, (3) ischemic acute kidney injury treated with ischemic preconditioning and L-NAME. Six animals were provided for each group (total animals: $n=18$).

For the measurement of renal sympathetic nerve activity, uninephrectomized rats were anesthetized with pentobarbital (50 mg/kg, i.p.). Renal sympathetic nerve activity was recorded from the left renal nerve branch before and during the 45-min ischemic period, as previously described by Shokoji et al. (2003). The nerve was isolated near the aortic-renal arterial junction through a left flank incision and placed on a Teflon-coated stainless-steel bipolar electrode. The renal nerve and electrode were covered with silicone rubber. The renal nerve discharge was amplified using a differential amplifier (AVB-11A, Nihon Kohden, Osaka, Japan) with a band-pass filter (low frequency, 50 Hz; high frequency, 1 kHz). The amplified and filtered signal was visualized on a dual-beam oscilloscope (VC-11; Nihon Kohden) and monitored by an audio speaker. The output from the amplifier was integrated by an integrator (EI601G; Nihon Kohden) with 1-s resetting. The output from the integrator was recorded and analyzed with PowerLab (ML750; ADInstruments, Castle Hill, Australia). For the quantification of renal sympathetic nerve activity, the height of integrated nerve discharge was measured for 30 s in each experiment. The changes in nerve activity were expressed as percentages of the resting spontaneous nerve activity in the rats with or without ischemic preconditioning.

2.3. Analytical procedures

Blood urea nitrogen and plasma creatinine levels were determined using a commercial kit, the BUN-test-Wako and Creatinine-test-Wako (Wako, Osaka, Japan), respectively. Urinary osmolality was measured by freezing point depression (Fiske Associates, Norwood, MA, USA).

Norepinephrine concentration in renal venous plasma was measured by high-performance liquid chromatography with an amperometric detector (HTEC-500; EICOM, Kyoto, Japan), as previously reported (Hayashi et al., 1991).

2.4. Histological studies

Excised left kidneys were processed for light microscopic observation, according to standard procedures. The kidneys were preserved in phosphate-buffer 10% formalin, after which the kidneys were chopped into small pieces, embedded in paraffin wax, cut at 4 μm, and stained with hematoxylin and eosin. Histopathological changes were analyzed for tubular necrosis, proteinaceous casts, and medullary congestion, as described by Caramelo et al. (1996). Tubular necrosis and proteinaceous casts were graded as follows: no change (0), mild (1; unicellular, patchy isolated damage), moderate (2; damage less than 25%), severe (3; damage between 25% and 50%), and very severe (4; more than 50% damage). The degree of medullary congestion was defined as no congestion (0), mild (1; vascular congestion with identification of erythrocytes by ×400 magnification), moderate (2; vascular congestion with identification of erythrocytes by ×200 magnification), severe (3; vascular congestion with identification of erythrocytes by ×100 magnification), and very severe (4; vascular congestion with identification of erythrocytes by ×40 magnification). Evaluations were made by an observer who was blind to the treatment origin of the tissue.

2.5. Drugs

L-NAME was purchased from Tokyo Chemical Industry (Tokyo, Japan). It was dissolved in saline (0.9%). Other chemicals were obtained from Nacalai Tesque (Kyoto, Japan) and Wako Pure Chemical Industries.